Orientation Tuning and Contrast Dependence of Continuous Flash Suppression in Amblyopia and Normal Vision

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Submitted: January 25, 2018
Accepted: October 13, 2018

Citation: Gao TY, Ledgeway T, Lie AL, et al. Orientation tuning and contrast dependence of continuous flash suppression in amblyopia and normal vision. *Invest Ophthalmol Vis Sci*. 2018;59:5462–5472. https://doi.org/10.1167/iovs.18-23954

Purpose. Suppression in amblyopia may be an unequal form of normal interocular suppression or a distinct pathophysiology. To explore this issue, we examined the orientation tuning and contrast dependence of continuous flash suppression (CFS) in adults with amblyopia and visually normal controls.

Methods. Nine patients (mean age, 26.9 ± SD 4.7 years) and 11 controls (mean age, 24.8 ± SD 5.3 years) participated. In the CFS paradigm, spatially one-dimensional noise refreshing at 10 Hz was displayed in one eye to induce suppression of the other eye, and suppression strength was measured by using a grating contrast increment detection task. In experiment 1, noise contrast was fixed and the orientation difference between the noise and the grating was varied. In experiment 2, noise and grating orientations were identical and noise contrast was varied.

Results. Suppression patterns varied in both groups. In experiment 1, controls showed consistently orientation-tuned CFS (mean half-height bandwidth, 35.8° ± SD 21.5°) with near-equal strength between eyes. Five of nine patients with amblyopia exhibited orientation-independent CFS. Eight patients had markedly unequal suppression between eyes. Experiment 2 found that increasing the noise contrast to the amblyopic eye may produce suppression of the fellow eye, but suppression remained unequal between eyes.

Conclusions. Our data revealed that orientation specificity in CFS was very broad or absent in some patients with amblyopia, which could not be predicted by clinical measures. Suppression was unbalanced across the entire contrast range for most patients. This suggests that abnormal early visual experience disrupts the development of interocular suppression mechanisms.

Keywords: continuous flash suppression, interocular suppression, amblyopia, psychophysics

Disruptions to visual experience early in life can cause abnormal cortical visual processing, a condition known as amblyopia. In humans, amblyopia is most commonly caused by strabismus (spatially decorrelated inputs) and/or anisometropia (monocular blur).[1] Unilateral amblyopia causes a range of visual impairments, including reduced monocular visual acuity, reduced contrast sensitivity, poor stereoacuity, and deficits in higher cortical visual processing (e.g., global motion coherence or face perception).[2,3] Patients also demonstrate suppression of the amblyopic eye by the nonamblyopic fellow eye during normal viewing. Greater imbalances in interocular suppression are correlated with greater interocular differences in visual acuity and worse stereoacuity.[4–9] Unbalanced interocular suppression in amblyopia has been advocated as a target mechanism for binocular therapy, with the rationale that rebalancing interocular suppression may improve visual functions.[10–12]

In animals with experimentally induced amblyopia, ocular dominance in the visual cortex favors the fellow eye,[13,14] interocular suppression appears to increase relative to binocular excitation,[15–17] and normal orientation matching of inputs from the two eyes are disrupted.[18,19] In addition, interocular suppression appears less orientation selective[16] and phase selective[17] than in controls.[20] Orientation- and phase-specific binocular responses are prerequisites for binocular summation and stereopsis.[21] In clinical practice, children undergoing amblyopia treatments are often left with subnormal stereoacuity even after monocular deficits are ameliorated.[22] It is possible that in these cases, abnormal suppression is a cortical limitation to good binocular vision.

In binocularly normal individuals, interocular suppression can be induced by the presentation of dichoptic stimuli designed to produce binocular rivalry or contrast masking. The strength of interocular suppression can be quantified by measuring contrast detection thresholds for a probe presented to the suppressed eye.[23–27] In the normal visual system, suppression is strongest when the probe and suppressor stimuli share similar orientations and weakens in nonparallel

orientations, resulting in an orientation-tuning function for suppression with the same shape as the typical orientation-tuning function of V1 cells.[23,26,28]

A lack of orientation-tuned interocular suppression in amblyopia would suggest the presence of a suppression mechanism that is qualitatively different from the mechanism operating in normal vision, and may indicate a need for specifically targeted therapies. It has been reported that suppression of the amblyopic eye is orientation tuned in anisometropic amblyopia,[24,29] less orientation tuned in unilateral strabismus (often associated with amblyopia),[29,30] and orientation independent in alternating strabismus (generally not associated with amblyopia).[29] However, orientation independent suppression regardless of amblyopia etiology has also been reported.[27] These studies are difficult to compare directly owing to methodologic and participant differences. This motivated us to perform the current study using a recently developed technique called continuous flash suppression (CFS).[31]

CFS occurs when a dynamic pattern presented to one eye renders an image in the other eye invisible for up to several minutes. Unlike other suppression-induction techniques such as binocular rivalry, perceptual dominance does not easily alternate between the two eyes. Therefore, CFS allows for more precise control of which eye is suppressed at any one time. In addition, the sustained suppression induced by CFS is more similar to suppression in amblyopia than the alternating suppression induced by binocular rivalry. In visually normal subjects, CFS magnitude can be quantified by measuring elevations of detection threshold for a probe stimulus in the other eye.[25,32] This is based on the assumption that suppression reduces sensitivity to visual stimuli. CFS is thought to have a feature-selective component and a tonic component. The feature-selective component strengthens when the suppressor stimulus and the probe are similar in spatial frequency,[33] chromaticity,[34] temporal frequency,[35] and orientation.[33] The tonic component is unaffected by these manipulations.

We used CFS to investigate the spatial properties of interocular suppression in adults with amblyopia and visually normal controls. Our aim was to examine whether suppression in amblyopia is a simple imbalance between eyes, where orientation tuning would be preserved, or whether distinct pathophysiology may be involved. In experiment 1, we presented a spatially one-dimensional, dynamic noise mask with a fixed mean contrast to one eye and varied its orientation relative to a fixed-orientation probe grating presented to the other eye, to investigate whether CFS was orientation independent in patients with amblyopia. We observed that in most patients, the amblyopic eye was not able to suppress the fellow eye at the noise mask contrasts used. In experiment 2, we examined the contrast dependence of CFS in patients and controls to investigate whether it was possible for the amblyopic eye to suppress the fellow eye at higher CFS noise contrasts.

Methods

Participants

All participants were between 20 and 40 years of age. Eleven patients with unilateral amblyopia associated with anisometropia and/or strabismus were screened. Amblyopia was defined as an interocular difference in best-corrected visual acuity of more than 0.10 logMAR (one line), at least 0.00 logMAR (20/20) visual acuity in the fellow eye, and a history of anisometropia and/or strabismus. Two patients with incomitant strabismus were ineligible owing to inability to maintain stable alignment during dichoptic viewing. Thus nine patients (mean age, 26.9, ± standard deviation [SD] 4.7 years) completed the first experiment. All but one of these patients had previous treatment for amblyopia. Eleven control subjects participated (mean age, 24.8 ± SD 5.3 years, including three authors: TYG, ALL, and NA, with the remainder being naïve to the experiment). Controls had 0.00 logMAR (20/20) or better corrected visual acuity in each eye, 40 arcsec on the Randot Preschool Stereoacuity Test, and no history of eye disease or binocular vision problems. One additional subject (SW) with successfully treated childhood strabismic amblyopia also took part. This patient met the visual acuity and stereoacuity criteria for the control group, but had intermittent alternating exotropia. The Table details the clinical characteristics and previous treatment histories of the patients and SW.

Written informed consent was given by all participants. The study was approved by The University of Auckland Human Participants Ethics Committee and was conducted in accordance with the tenets of the Declaration of Helsinki.

Clinical Measurements

Clinical testing included assessment of refractive error (retinoscopy and subjective refraction), best-corrected monocular visual acuity (electronic Early Treatment of Diabetic Retinopathy Study test at 3 m[36]), near stereoacuity (Fly Stereo Acuity Test with LEA Symbols, Vision Assessment Corporation, Elk Grove Village, IL, USA; Randot Preschool Stereoacuity Test, Stereo Optical Co.,[37] Chicago, IL, USA; TNO stereoacuity test, 16th edition, Laméris Ootech BV, Ede, The Netherlands), cover test, visuoscopy, and Worth 4-dot test at 40 cm and 6 m. Every participant completed all tests while wearing optimal refractive correction (habitual or trial lenses).

Psychophysics

Sensory Eye Dominance. For controls, sensory eye dominance was determined by using dichoptic global motion coherence.[38] Random dot kinematogram stimuli were displayed on a gamma-corrected ASUS VG278 monitor with a 120-Hz frame-sequential stereo display (Beitou District, Taipei, Taiwan), viewed from 60 cm through LCD shutter glasses. Each measurement included two interleaved staircases for dichoptic global motion coherence thresholds, one with signal dots shown to the left eye and noise dots to the right eye, and the other with the opposite configuration. The eye with the lower global motion coherence threshold (i.e., requiring less signal dots to discriminate motion direction) was considered the dominant eye. Measurements were repeated at least twice to confirm eye dominance.

Interocular Suppression Measured by Using Dichoptic Global Motion. All patients and controls completed an established, two-step dichoptic global motion measure of suppression[39–41] for comparison with CFS. Stimuli were presented by using the apparatus described above. The first step involved measurement of a binocular global motion coherence threshold using a two-alternative forced choice (2-AFC) up-versus-down motion discrimination task. In the second step, the threshold number of signal dots from step one was shown to the amblyopic/nondominant eye at a fixed contrast, and the remaining noise dots were shown to the fellow/dominant eye with a variable contrast. The fellow/dominant eye noise dot contrast required to interfere with perception of signal dot motion (such that discrimination of motion direction was at 75% correct) was determined by the average of five staircases. A dichoptic contrast ratio (fellow/dominant eye noise dot contrast threshold divided by amblyopic/nondominant eye signal dot contrast) was then

Table. Clinical Characteristics of Patients

Observer	Age, y	Sex	Associated Clinical Classification	Refraction	VA, logMAR	Stereoacuity, arcsec			Strabismus	Worth 4-Light		History of Previous Treatments	Noise Contrast in Exp 1	Participated in Exp 2
						Randot	TNO	Fly		40 cm	6 m			
RL	27	F	Mixed	R +0.75/−1.00x112 L +3.75/−1.00x75	−0.10 1.02	Nil	Nil	Fly only, 3000–4800	2 L ET manifest 4Δ nasal EF in left eye.	Fusion	L suppression	Detected 5 yo, glasses and part-time patching 5–6 yo.	0.25	No
MCR	23	M	Strabismic	R +0.75 L Plano	0.28 −0.18	400	480	100	Primary microtropia 0.5-1Δ R nasal EF	Fusion	Fusion	Detected before age 5 yo, glasses full-time age 5–15 yo, patching 6 h/d age 5–8 yo.	0.30	Yes
RM	25	F	Mixed	R −2.50 L +4.50/−1.50x60	−0.16 0.30	Nil	Nil	63	6 L XT at near 10 L XT 2 hyperT at distance	Fusion	Fusion	Detected age 4 yo, glasses age 4–8 yo, patching 12 h/d 4–8 yo. Also tried atropine.	0.10	No
HD	22	F	Anisometropic	R +0.25 L +7.00/−1.25x50	−0.22 0.40	800	Nil	40	None	Fusion	L suppression	Detected 5 yo, glasses full-time 5–7 yo, patching 12 h/d 5–6 yo.	0.10	Yes
BW	22	M	Anisometropic	R Plano/−0.25x10 L +2.75/−0.25x25	−0.24 0.34	100	240	40	None	Fusion	Fusion	Detected 4 yo, patching 1 h/d between 5–6 yo and 7–8 yo. Glasses first worn 3 months ago.	0.20	Yes
LM	33	F	Anisometropic	R +0.25/−0.75x105 L +3.00/−0.50x50	−0.30 0.20	60	240	50	None	L suppression	Fusion	Detected 5 yo, glasses full-time and patching part-time 5–6 yo. Also tried atropine.	0.30	Yes
CC	32	F	Anisometropic	R −1.00/−0.75x157 L −3.50/−3.50x180	−0.26 0.20	60	60	20	None	Fusion	Fusion	Detected 6 yo, no treatment.	0.10	No
KM	32	F	Anisometropic	R −4.75/−1.25x115 L +0.25/−0.25x175	0.24 −0.10	100	240	32	None	Fusion	Fusion	Detected 13 yo, glasses full-time since, additional treatments not offered.	0.30	No
LW	21	F	Anisometropic	R +0.75/−0.25x135 L +4.00/−1.00x75	−0.08 0.10	200	120	100	None	Fusion	Fusion	Detected 5 yo, glasses full-time 5–15 yo, then part-time. Patching part-time 5–6 yo.	0.30	Yes
SW	22	F	Previous strabismic amblyopia	R +0.25/−0.75x110 L -0.25/−0.25x45	−0.10 −0.14	40	30	25	15–20 intermittent alternating XT at near and distance.	Alternating suppression when manifest, R dominant. Fusion when deviation latent.		Detected before 4 yo, surgery for L ET at 4 yo, patching 4–5 yo. No significant refractive error, never wore glasses.	0.30	No

Where "none" is listed under strabismus, the participant had no manifest strabismus on cover test and also did not show eccentric fixation on visuoscopy. Angles of strabismus are given in prism diopters. EF, eccentric fixation; ET, esotropia; Exp, Experiment; hyperT, hypertropia; Fly, Fly Stereo Acuity Test; L, left eye; logMAR, logarithm of the minimum angle of resolution; R, right eye; Randot, Randot Preschool Test; TNO, TNO stereoacuity test; VA, visual acuity; XT, exotropia; yo, years old.

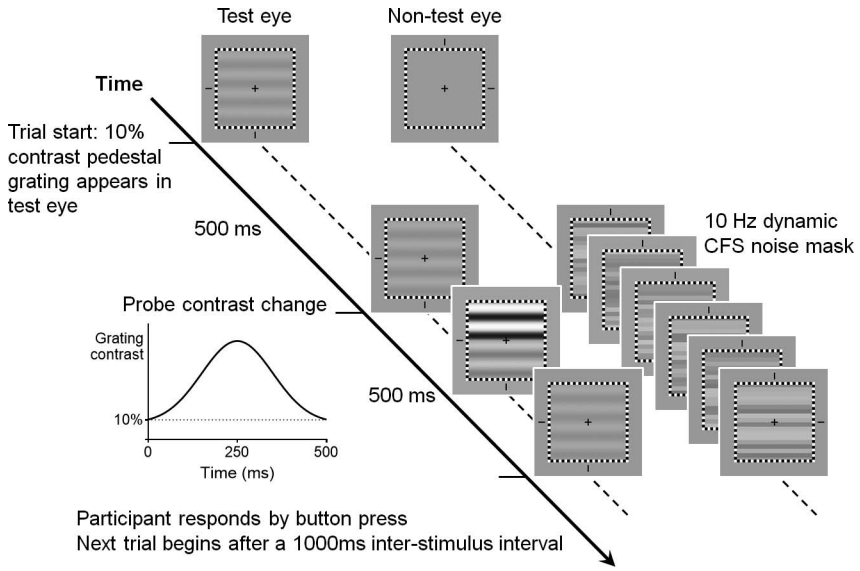

Figure 1. Schematic of continuous flash suppression stimuli. A 0° noise mask orientation is depicted. On each trial, the participant indicated whether the top or bottom of the fusion square changed in contrast (2-AFC). The contrast increment threshold measured in the test eye represented the stimulus strength required to break through suppression generated by the noise mask.

calculated for each participant. A ratio of 1.0 indicated that no interocular contrast difference was required for equal performance between the eyes. Lower values indicated greater suppression of the amblyopic/nondominant eye relative to the fellow/dominant eye.

Continuous Flash Suppression. CFS stimuli were displayed on two synchronized, gamma-corrected Samsung SyncMaster 2233RZ monitors (Suwon, Gyeonggi-do, South Korea) viewed through a mirror stereoscope at a distance of 2.35 m. The participant's head was stabilized by using a chin rest, and stereoscope mirrors were individually aligned for each participant by using alternating cover tests. Stimuli were displayed inside a square fusion frame (2.57°), with a central fixation cross and peripheral fusion check lines. On each trial (Fig. 1) the test eye saw a horizontally oriented 1.6 cyc/deg pedestal grating at 0.10 Michelson contrast (contrast refers to the ratio of presented contrast to the maximum displayable contrast). The phase of this grating was always 0° (i.e., ± sine phase) with respect to the horizontal midline and each half of the square display area contained two whole cycles, preventing luminance edge artefacts. Whether the grating was a positive sine wave or negative sine wave was randomized on each trial. During each trial, a smooth contrast increment occurred in either the top or the bottom half of the square with a Gaussian temporal profile. Participants indicated the location of the contrast increment in a 2-AFC protocol. A three-down-one-up staircase was used to measure the increment detection threshold corresponding to 79.3% accuracy. A proportional step size of 30% was used before the fourth reversal of the staircase and 15% thereafter. Each staircase terminated after eight reversals and the last four were averaged to calculate threshold.

The nontested eye viewed either the mean luminance screen with only the fusion border (the "monocular" condition) or a one-dimensional, spatially broadband noise mask refreshing at 10 Hz to induce CFS. The noise mask was composed of 0.15° width stripes (Fig. 1). The luminance of each stripe was assigned by random sampling with replacement from a uniform probability distribution spanning a range determined by an assigned Michelson contrast limit, hereafter referred to as the "noise contrast" for brevity. When CFS was induced, the test-eye threshold represented the contrast increment required to overcome suppression within at least a portion of the stimulus aperture, such that the position of the contrast increment (up versus down) could be located.

The test probe grating was always horizontal. Noise mask orientation in experiment 1 varied from 0° (horizontal, parallel to the probe grating) to 90° (vertical, orthogonal to the probe grating) in 15° steps.

Before CFS data collection, participants practiced all stimulus presentation conditions for the 0° and 90° noise mask orientations. Noise mask contrast was initially set at 0.30 for all participants. Five patients could not perform the probe task with their amblyopic eye when the 0.30 contrast noise mask was shown to their fellow eye owing to complete suppression (Table). In these patients, noise mask contrast was lowered in 0.05 steps until increment thresholds became measurable. This reduced noise contrast was then used for data collection in both eyes of that patient. All controls used 0.30 noise contrast in experiment 1.

Each participant completed four to five blocks of testing. Each block contained one staircase of every combination of eye and noise mask orientation plus the monocular thresholds for each eye (16 staircases) in a random order. Each block took approximately 60 minutes (including regular breaks). Testing sessions occurred across multiple days.

Statistical Analyses

Analyses were performed with SPSS Statistics Version 23 (IBM, Armonk, NY, USA), with $P \leq 0.05$ as the criterion for statistical significance. Results are reported as mean ± SD. Parametric tests were used for data that did not violate assumptions of normality (assessed by using the Shapiro-Wilk test $P > 0.05$), otherwise nonparametric tests were used.

Monocular contrast increment thresholds were analyzed by using 2-way mixed analysis of variance (ANOVA), with a between-subjects factor of group (controls versus patients) and a within-subjects factor of eye (dominant/fellow versus nondominant/amblyopic).

Suppression induced by CFS was calculated in threshold elevation units by dividing increment detection thresholds during CFS by monocular thresholds for the same test eye. This normalization accounted for any monocular deficits in contrast

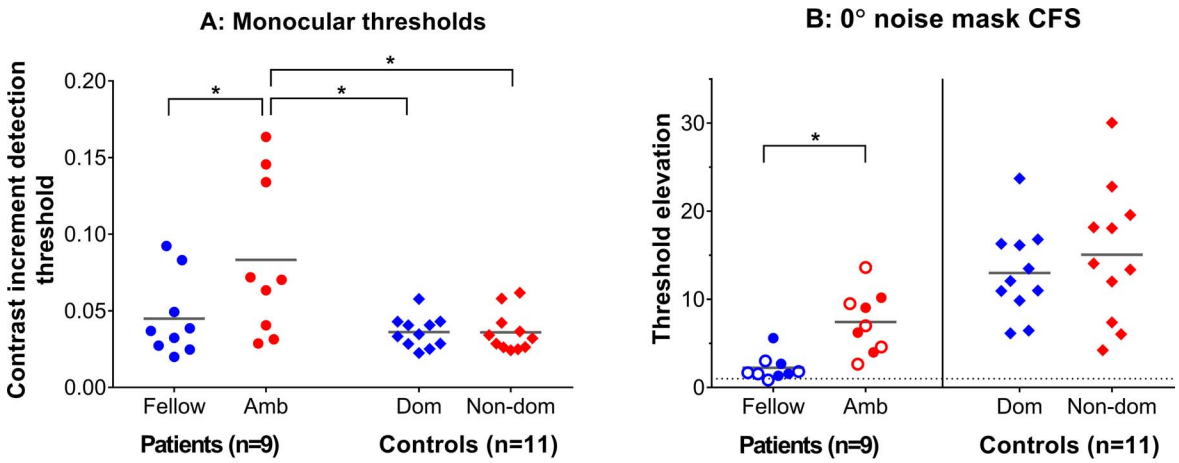

FIGURE 2. Monocular contrast increment thresholds (**A**) and threshold elevations induced by CFS with a 0° orientation difference between the noise and probe grating (**B**) for each eye of patients and controls. Threshold elevations are calculated by dividing contrast increment threshold measured with CFS by the monocular thresholds for the same test eye. *Symbols* indicate individual participants and *horizontal lines* indicate group means for the fellow and amblyopic (Amb) eyes of patients, and the dominant (Dom) and nondominant (Non-dom) eyes of controls. *Asterisks* indicate statistically significant differences. In (**B**), *filled symbols* indicate participants tested with 0.30 noise mask contrast, and *open circles* indicate patients for which noise mask contrast was reduced to 0.10 to 0.25. Every participant was always tested with the same noise mask contrast in both eyes, so suppression strength between eyes was compared, but comparisons were not made between the patient and control groups.

increment detection. The distributions of threshold elevations for the two eyes were then compared with the nonparametric Wilcoxon signed rank test at each noise mask orientation to examine whether suppression was equal between eyes. This analysis was performed separately for the patients and the controls because the lowered noise mask contrast used for some patients prevented us from directly comparing CFS thresholds between the two groups.

We examined orientation tuning as a binary outcome for each eye of every participant. Threshold elevation data were fitted with two functions with GraphPad Prism Version 7.03 (GraphPad Software, Inc., San Diego, CA, USA), and the best-fit model was determined by using the extra sum-of-squares *F* test. The null hypothesis for each test was that threshold elevation (the dependent variable, y) did not vary with noise orientation (the independent variable, x), modelled by a simple horizontal line with the following equation:

$$y = c, \quad (1)$$

where c is a constant. The alternative hypothesis was orientation-tuned suppression, represented by a one-sided Gaussian function peaking at a noise orientation of 0° (horizontal):

$$y = \exp\left(-(x/b)^2 \ln 2\right)a + c, \quad (2)$$

where a is the amplitude (difference between maxima and minima) and represents the amount of orientation-specific suppression; b is the half-height half-width orientation-tuning bandwidth; and c is a constant representing the tonic component of suppression. If the null hypothesis was rejected ($P \leq 0.05$), suppression for that eye was considered orientation tuned and the tuning bandwidth was estimated from the best-fitting function parameters. If the null hypothesis was not rejected, suppression was considered to be orientation independent.

Finally, to investigate if the clinical characteristics of the nine patients with amblyopia were significantly associated with their performance on psychophysical measures, Spearman rank correlations were used to assess the relationships among interocular difference in visual acuity, Randot Preschool Test stereoacuity, dichoptic global motion contrast ratio, amblyopic eye contrast increment thresholds, and CFS threshold elevations for the amblyopic eye using the 0° noise mask orientation (maximum suppression). Nil stereopsis was assigned an arbitrary, conservative value of 10,000 arcsec in this nonparametric analysis.[42]

RESULTS

Monocular Contrast Increment Discrimination

Monocular contrast increment thresholds are shown in Figure 2A. A 2-way mixed ANOVA was used for analysis, as the data passed the Shapiro-Wilk test for normality ($P > 0.05$). As expected, there was a significant interaction between eye and group ($F(1, 18) = 12.08$, $P = 0.003$). Amblyopic eyes had higher mean increment thresholds than fellow eyes ($F(1, 8) = 12.70$, $P = 0.007$), whereas dominant and nondominant eyes of controls did not differ in mean contrast sensitivity ($F(1, 10) = 0.44$, $P = 0.52$). These results were confirmed with nonparametric tests and parametric testing of log-transformed data.

Strength of Continuous Flash Suppression, Dichoptic Global Motion Suppression, and Clinical Amblyopia Severity

Figure 2B shows threshold elevations measured with CFS when the noise mask was oriented at 0°, after normalizing for monocular performance. When a 0.30 contrast noise mask was used, suppression of the amblyopic eye in patients was stronger than suppression in controls. For five of nine patients, suppression was so strong that the probe contrast increment remained invisible at maximum contrast (0.9), preventing threshold measurements. Therefore, a reduced noise mask contrast was used for these five patients to lower suppression to a measurable range (Fig. 2B, open circles). The threshold elevation metric we used accounted for elevated monocular increment detection thresholds. This meant that the maximum measurable threshold elevation for each eye was equal to the maximum probe contrast increment (0.9) divided by the monocular threshold. Thus, higher monocular thresholds restricted the maximum amount of suppression that could be measured by CFS in some patients.

In patients, the group distributions of CFS threshold elevations for amblyopic eyes were higher than for fellow eyes at the 0° noise mask orientation (Fig. 2B; Wilcoxon $Z = 2.67$, $P = 0.008$). In controls, group distributions for dominant and nondominant eyes were not statistically significantly different (Fig. 2B; Wilcoxon $Z = 1.69$, $P = 0.09$). Similar results were found at all other noise orientations (patients: Wilcoxon $Z \geq 2.67$, $P < 0.02$; controls: all Wilcoxon $Z \leq 1.69$, $P > 0.09$), though there was substantial individual variation (Fig. 3). On the dichoptic global motion test for suppression, patients showed on average more unbalanced suppression than controls (dichoptic contrast ratios: patients 0.60 ± 0.24, controls 0.83 ± 0.24; unpaired $t_{18} = 2.14$, $P = 0.046$; Supplementary Fig. S1).

For the nine patients with amblyopia, greater interocular imbalance on dichoptic global motion was significantly correlated with worse amblyopic eye monocular contrast increment threshold (Spearman $r_s = -0.82$, $P = 0.007$; Supplementary Fig. S2). No significant correlations were found between CFS threshold elevations for the amblyopic eye and dichoptic global motion suppression, nor between these two suppression measures and any clinical measures (Spearman $r_s = -0.20$ to 0.53, all $P > 0.11$; Supplementary Fig. S2).

Orientation Tuning of Continuous Flash Suppression

Individual participant CFS data are shown in Figure 3. Controls exhibited orientation-tuned suppression. In general, the strength of suppression was similar between the two eyes, although this did vary across individuals. Mean tuning bandwidth for all controls (except LZ, where tuning was broad) was 35.8 ± SD 21.5°.

Six patients (RL, RM, HD, LM, CC, and KM) showed little or no suppression of their fellow eye by the amblyopic eye at any orientation, and thus orientation tuning of suppression could not be reliably examined for their fellow eyes. Suppression of the amblyopic eye by the fellow eye was found for all patients. For amblyopic eyes, six patients showed orientation-independent suppression and three showed orientation tuning. The amplitudes of orientation-tuned suppression were small for HD and MCR, while tuning curves for patients BW and LW resembled those for control subjects. Of the six patients with anisometropia, three showed orientation-tuned amblyopic eye suppression (HD, BW, LW) and three showed orientation-independent amblyopic eye suppression (LM, KM, CC). Thus, the absence of strabismus did not reliably predict orientation-tuned suppression. There was also no apparent relationship between the presence of orientation-tuned suppression and stereoacuity on any of the three clinical tests used.

Although both eyes of each participant were tested with the same physical noise mask contrast, eight of the nine patients demonstrated unequal suppression between the eyes. The exception was patient LW, who had the mildest amblyopia (interocular visual acuity difference of 0.18 logMAR) amongst the nine patients and demonstrated a suppression profile similar to that of control participants. As the CFS noise mask contrast was lowered for five patients to enable measurable suppression, the difference in suppression strength (i.e., threshold elevations on the *y*-axes) between these five patients and the other subjects cannot be directly compared.

Continuous Flash Suppression in Successfully Treated Amblyopia

Subject SW had strabismic amblyopia and achieved clinically normal monocular visual acuity and stereoacuity following childhood treatments, but had intermittent alternating strabismus (Table). Figure 4A shows her monocular contrast increment thresholds compared to the group means and ranges replotted from Figure 2. SW had approximately equal monocular contrast increment detection thresholds in the two eyes, but thresholds were slightly higher (worse) than control participants. CFS strength was equal between eyes, but suppression was clearly not orientation tuned (Fig. 4B), indicating that this aspect of SW's binocular vision did not completely normalize after childhood amblyopia treatments.

Experiment 2: Contrast Dependence of Continuous Flash Suppression

In the first experiment, six patients with amblyopia (RL, RM, HD, LM, CC, and KM) showed little or no suppression of the fellow eye when dynamic noise was presented to their amblyopic eye at the contrasts tested. This may be because interocular suppression was entirely unidirectional in their visual system, or perhaps the CFS noise mask was not of sufficient contrast intensity to activate suppression of the fellow eye by the amblyopic eye. To examine this issue, we investigated the contrast-dependent properties of CFS in Experiment 2.

Methods. Experiment 2 was completed by five patients from experiment 1 (Table) and three controls (author TYG, naïve participants CS and LG). The probe contrast increment task was the same as that used in experiment 1. The orientation of the CFS noise mask was fixed at 0° (identical to that of the grating stimulus) to maximize induced suppression. Noise contrast was varied from 0.10 to 1.0. Each participant completed four to five blocks of threshold measurements. Each block contained all testable noise contrast levels for both eyes, presented in a random order. Thresholds were normalized for monocular performance in the same manner as experiment 1. We have previously shown that the change in contrast increment threshold with increasing noise contrast is well characterized by a straight line on linear-log axes,[43] thus the results for each eye were fitted with a semi-log function of the form:

$$y = a\log_{10}(x) + b, \quad (3)$$

where a is the slope of the function and b is the intercept.

Results. Figure 5 shows the contrast dependence of CFS threshold elevations for each participant. Patients LW, BW, and MCR showed suppression of the fellow eye by the amblyopic eye during experiment 1. In experiment 2, increasing noise contrast in their amblyopic eye increased suppression of their fellow eye, but suppression remained unequal between eyes at all noise contrasts tested. Patients LM and HD did not show suppression of the fellow eye during experiment 1. In experiment 2, LM showed relatively weak suppression of the fellow eye, but only when the noise mask contrast in the amblyopic eye was close to 1.0. Patient HD had the most severe amblyopia in this experiment, and increasing the contrast of the noise mask presented to her amblyopic eye had no effect on fellow eye performance. Despite this, HD reported that the noise mask presented to the amblyopic eye was always visible on top of the probe grating, and HD could accurately report its orientation with both eyes open. This suggested that mask visibility was not responsible for this effect. Increasing noise contrast above 0.10 in HD's fellow eye caused complete suppression of the amblyopic eye, rendering the probe task impossible to complete. This result suggests that suppression induced by this CFS paradigm may indeed be unidirectional for patient HD.

In controls, suppression gain with increasing noise contrast (i.e., the slope parameter a of Equation 3) was nearly equal

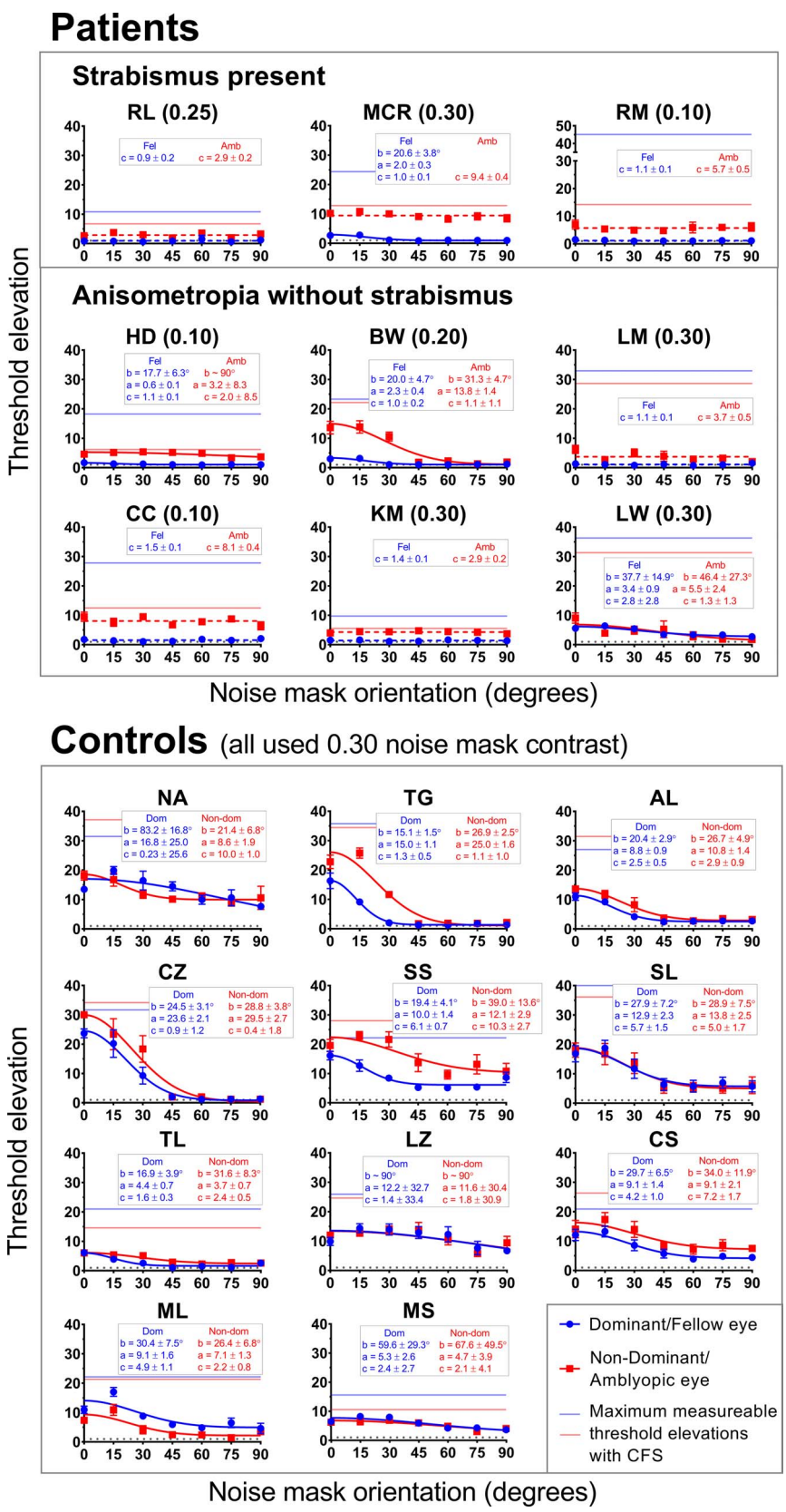

FIGURE 3. Individual results for CFS-induced threshold elevations as a function of noise mask orientation. The extra sum-of-squares F test (see Methods) was used to determine whether orientation tuning was present (*solid lines*) or absent (*dashed lines*) for each eye. Fitting parameter estimates ± standard errors are indicated in inset panels. Where orientation tuning was present, the half-Gaussian fit parameters are as follows: b = bandwidth, a = amplitude, and c = tonic suppression. Where orientation tuning was absent, the fitted parameter for the horizontal line is c = tonic suppression. For patients, the noise mask contrast used for their two eyes is indicated in the graph titles. Each data point represents the mean of

four to five threshold measurements. *Error bars* are 1 standard error of the mean (may be smaller than data point). *Horizontal gray dotted lines* indicate a threshold elevation of 1, where the noise mask did not produce enough suppression to affect probe task performance. *Horizontal solid lines* indicate the maximum measureable threshold elevations with CFS for the dominant/fellow eyes (*blue*) or nondominant/amblyopic eyes (*red*), which varied according to monocular contrast increment thresholds.

between the two eyes of each subject but differed between individuals.

Discussion

Using CFS, we found that central visual field interocular suppression was unequal between the eyes in most patients with amblyopia, and that suppression in a subset of patients was not orientation tuned. This indicates that suppression in some patients with amblyopia is less feature selective than in controls. Unlike a previous report,[29] we found no relationship between presence of strabismus and orientation-independent suppression, but our data do support the notion that a range of suppression patterns are found in amblyopia.

Higher monocular increment detection thresholds in some of our patients limited the maximum threshold elevations that could be measured by CFS (Fig. 2). In addition, five of nine patients had very strong suppression and required reduced CFS noise contrasts to lower suppression into a measurable range. This produced the appearance of less suppression in the amblyopia group than the control group, though the methodologic differences mean that data from the two groups cannot be directly compared.

It is possible that limited measurable threshold elevations in the patient group masked subtle orientation tuning, particularly in patients who were close to the measurable limit of suppression on CFS. However, we note that the patients who showed similar threshold elevations as controls and had sufficient measurement range to detect orientation tuning (e.g., MCR, RM, and CC) still exhibited orientation-independent suppression of their amblyopic eyes.

Previous studies using dichoptic global motion,[4,44] phase matching,[5,45] and other contrast- or luminance-balancing suppression measures[6,8] report greater average suppression in patients with amblyopia than controls. However, these previously established methods (including the dichoptic global motion test used in this study) measure the interocular balance of suppression and do not account for monocular deficits. Our CFS method measured suppression of each eye separately, and our calculation of threshold elevations explicitly normalized for monocular deficits. The significant correlation we found between dichoptic global motion suppression and monocular contrast increment thresholds is consistent with previous work[46] and suggests that monocular deficits may be a key factor underlying measured imbalances in suppression. However, we note that CFS threshold elevations were still substantially more unbalanced between eyes in patients than controls even after monocular deficits were accounted for (Figs. 2B, 3, 5), thus supporting the existence of a substantial binocular imbalance in amblyopia.

Intriguingly, the four patients who were tested with the same 0.30 contrast noise mask as controls (Fig 3; MCR, LM, KM, LW) exhibited amblyopic eye threshold elevations in the lower range of controls. Recent animal studies have begun to elucidate the neural basis of interocular suppression, and there is some suggestion that in amblyopia, suppression of the amblyopic eye by the fellow eye is of a similar strength to interocular suppression in the normal visual cortex, while suppression of the fellow eye by the amblyopic eye is severely reduced or absent.[15,47] Human electrophysiology data have also suggested that amblyopia may be associated with a decrease in the total quantity of suppression in addition to the expected imbalance between eyes.[48] Our data are consistent with these findings. In addition, differences in the quantity of suppression in amblyopia do not explain the lack of orientation tuning we observed in some patients, which may be due to differences in suppression mechanisms.

The contribution of monocular orientation channels to the orientation tuning of interocular suppression also requires consideration. Previous studies have reported potentially broader orientation tuning or greater noise in monocular orientation channels for the amblyopic eye of some patients,[49,50] which may cause broader orientation tuning of interocular suppression. However, these studies use surround-suppression stimuli, which are not directly analogous to CFS because the mask and probe are spatially separated. In addition, the results of these previous studies show that monocular suppression in amblyopic eyes is orientation-tuned with either normal or slightly-broader-than-normal bandwidth, rather than the completely orientation-independent interocular

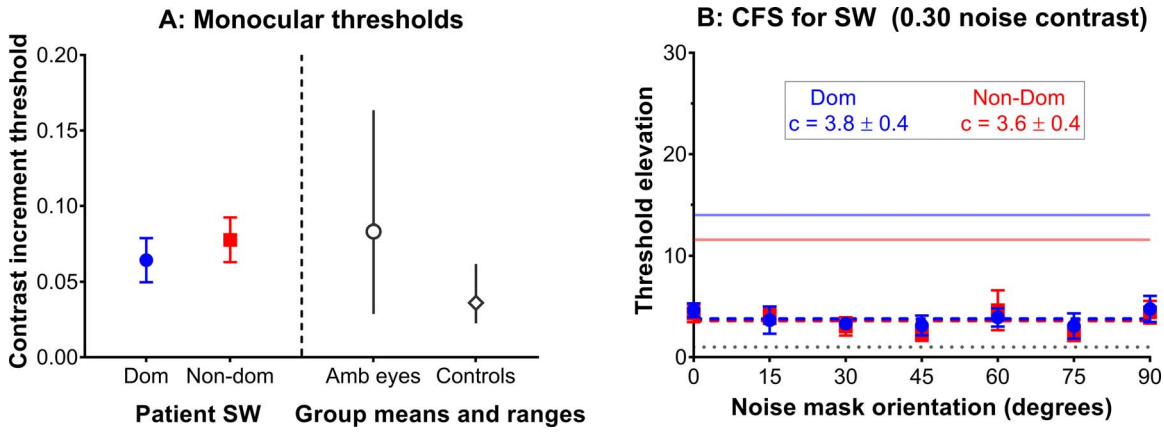

Figure 4. Monocular contrast increment detection thresholds (**A**) and threshold elevations induced by CFS as a function of noise mask orientation (**B**) for SW, a subject with successfully treated strabismic amblyopia. (**A**) *Left half*: Monocular thresholds for SW (mean and standard error of five measurements); *Right half*: Mean and range of monocular thresholds for amblyopic eyes of patients (Amb eyes) and both eyes of controls (Controls). (**B**) CFS was orientation independent in both eyes for SW (*dashed lines*). Graph labels follow the same conventions as Figure 5.

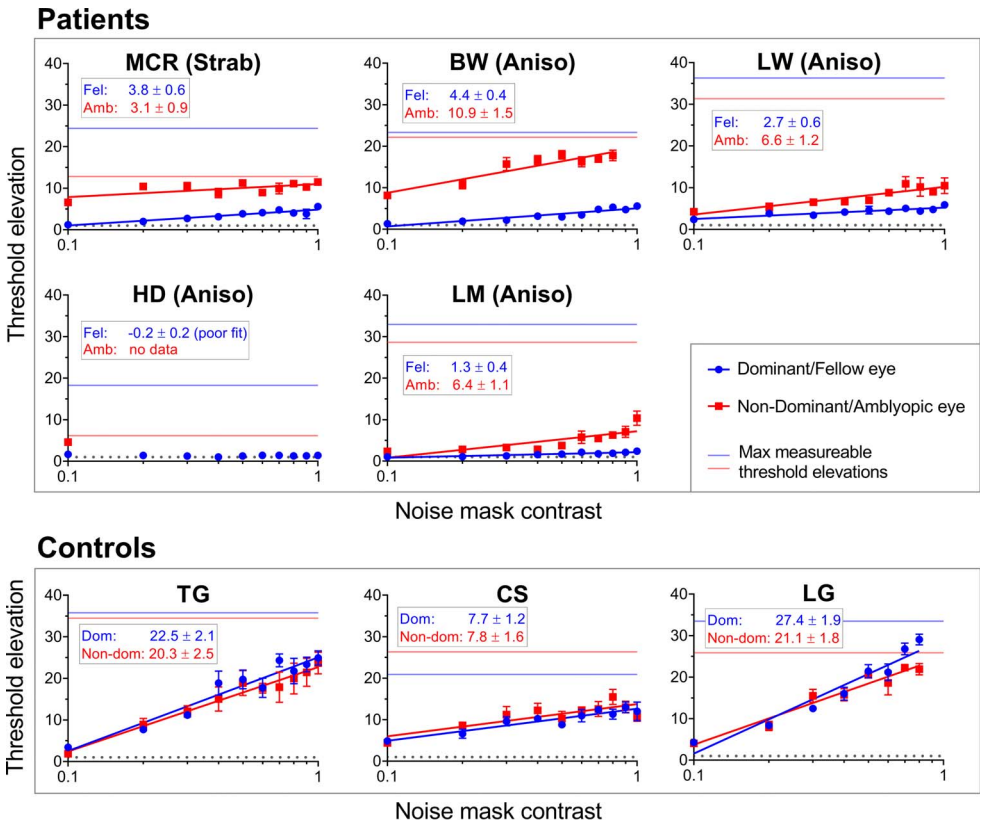

Figure 5. Threshold elevations induced by CFS as a function of noise mask contrast. The mean ± standard error for the slope parameter (*a*) of Equation 3 is provided for each eye in each panel. Each data point represents the mean of four to five threshold measurements. *Error bars* are 1 standard error of the mean (may be smaller than data point). *Horizontal gray dotted lines* indicate a threshold elevation of 1, where the noise mask had no suppressive effect, and *solid horizontal lines* indicate the maximum measureable threshold elevations for the dominant/fellow eyes (*blue*) and nondominant/amblyopic eyes (*red*). Missing data points for HD, BW, and LG were due to complete suppression at higher noise contrasts, rendering the probe grating invisible even at the maximum contrast increment.

suppression we found when using CFS. Nonetheless, the potential influence of monocular masking on dichoptic suppression measures requires further investigation.

Though we were unable to reliably and quantitatively compare suppression depth between participants, the same physical noise contrast was used for the two eyes of each participant so that whether suppression was equal or unequal between the eyes could be examined. Patient LW, who had mild amblyopia, showed equal suppression between eyes, which is consistent with previous studies reporting positive correlations between amblyopia severity and suppression strength.[4–9] Overall, however, we did not observe significant correlations between suppression of the amblyopic eye and clinical measures of amblyopia severity. The extensive testing time required in this study limited our sample size and CFS noise contrast also varied within the amblyopic group; therefore, the correlation results should be interpreted cautiously (see Supplementary Results for further details).

Our control subjects showed a surprising degree of variation in suppression characteristics. In experiment 1, four of 11 controls showed unequal suppression between eyes across multiple noise orientations (Fig. 3; TG, SS, CS, ML), consistent with previous studies showing strong eye dominance in 30% to 40% of the normal population.[38,51–53] In addition to this expected variation, threshold elevations in experiment 1 varied between participants by nearly 1 order of magnitude on the 90° noise mask and by a factor of 5 on the 0° noise mask. Some of the controls (particularly TG and CZ) showed threshold elevations close to unity at 60° to 90° orientations, indicating that noise masks of near-orthogonal orientation to the probe grating had minimal suppressive effects. In experiment 2, our three controls exhibited different suppression gains with increasing noise contrast (Fig. 5). Individual differences in susceptibility to CFS have been previously documented in visually normal subjects.[35,43] Our results expand on this and suggest that variation exists in both the tonic and feature-selective components of suppression, such that clinically normal observers may not behave uniformly on CFS tasks. The neural mechanisms underlying interocular suppression are still not fully understood, and future studies should aim to elucidate why this phenomenon exhibits such marked individual differences within the normal population as well as in patients with abnormal vision.

In a previous study using binocular rivalry in amblyopia, De Belsunce and Sireteanu[54] have found that the temporal duration of stimuli affects whether suppression of one image or superimposition is perceived. The effect of duration differs between individuals. In visually normal observers, the temporal structure of the dichoptic mask can also drive non–orientation-tuned suppression.[55] Our range of results were obtained by using a fixed duration CFS stimulus, and therefore the between-subject variability we observed may be related to individual differences in the time course of orientation-independent (generalized) and orientation-tuned (feature-selective) suppression. Studies using varying spatiotemporal stimulus configurations are required to address this possibility.

In summary, we showed that the potency of CFS depends critically both on low-level stimulus properties (e.g., contrast

and orientation) and the visual developmental history of the observer. In particular, we provided new evidence that interocular suppression in a subset of adults with amblyopia is less orientation selective than in visually normal observers, suggesting that suppression in amblyopia is not simply an extreme case of unbalanced normal interocular suppression. This type of orientation-independent suppression may represent additional cortical anomalies in binocular vision.

Acknowledgments

The authors thank all participants for their time.

Supported by funding from Postgraduate Research Student Support fund from the University of Auckland (TYG) and the Paul Dunlop Memorial Research Scholarship from the New Zealand Association of Optometrists (ALL). Equipment was provided by the School of Optometry and Vision Science, University of Auckland. During the period of this research study and preparation for publication, TYG received doctoral scholarship support from the University of Auckland and the New Zealand Association of Optometrists. PVM was supported by a research grant from The Leverhulme Trust. BT was supported by NSERC Grants RPIN-05394 and RGPAS-477166 and CIHR Grant 365136.

Disclosure: **T.Y. Gao**, None; **T. Ledgeway**, None; **A.L. Lie**, None; **N. Anstice**, None; **J. Black**, None; **P.V. McGraw**, None; **B. Thompson**, None

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